

# Optimizing the production of recombinant human Galectin-1: a general protocol for maintaining stability and lectin activity in the long run

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C. Abreu<sup>I</sup>, J. Kozák<sup>II</sup>, K. Hofbauerová<sup>III</sup>, V. Kopecký<sup>III</sup>, O. Vaněk<sup>I</sup>

<sup>I</sup>Department of Biochemistry, Faculty of Science, Charles University, Prague, Czech Republic, <sup>II</sup>Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic, <sup>III</sup>Institute of Physics, Faculty of Mathematics and Physics, Charles University in Prague, Prague, Czech Republic

Galectins are a family of lectins characterized by their binding affinity for  $\beta$ -galactosides and a shared amino acid sequence motif in their carbohydrate recognition domain. Galectins are involved in the modulation of inflammatory responses and mediation of immune suppressive mechanisms in tumour progression and evasion, making them attractive targets for research. Galectin-1 is comprised of a  $\beta$ -sandwich stabilized by a hydrophobic core and interactions at the dimer interface. Galectin-1 is highly susceptible to oxidative inactivation due to the presence of six cysteines in its sequence and their participation in the formation of intramolecular disulfide bonds that destabilize the folding of the protein, thus leading to the loss of its lectin activity [1].

Activity of Galectin-1 is dependent on the (i) correct folding of the carbohydrate-recognition domain, (ii) maintenance of the reduced state of its cysteines, and (iii) preservation of the spatial arrangement of the residues involved in ligand binding even in the carbohydrate-ligand-free form of the protein. With this in mind, we designed four constructs to be purified either by affinity chromatography on a lactosyl-sepharose column or by IMAC, and we characterized the protein variants in a time-dependent manner by SDS-PAGE, DLS, nanoDSF, SEC, CD, UV-Vis spectroscopy, and FP assays with high-affinity ligands, to establish a general protocol for the preparation of recombinant Galectin-1 for studies where long-term stability and maintenance of lectin activity are a requirement. Our findings show a clear improvement in the stability of the tag-less cysteine-to-serine mutant form of Galectin-1 over the remaining variants. The observations provided valuable insight into the factors relevant to maintaining the stability and lectin activity of Galectins.

[1] López-Lucendo MF et al. (2004) *J. Mol. Biol.* 343, 957-970

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